## THE REMARKS

Claims 1, 4, 5, 7-9, 13-16, 19, and 21-27 were pending prior to entering the amendments.

### The Amendments

Claim 1 is amended to clarify the meaning of the claim. The amendment of Claim 1 is supported by Publication 2005-0095605 at Paragraphs [0014], [108], [109], [0154], [158] and Figure 3.

The amendments of FIGURES 3-2 and 3-3 are to insert the bold "H". A marked-up copy is attached herewith to show the changes made. At Paragraph [0098], the application describes that FIGURES 3-1 to 3-8 depict an alignment of the amino acid sequences of 81 natural proteorhodopsin variants. The bold "**H**" indicates the position of a conserved histidine, which corresponds to H75 of Bac31A8. Applicants inadvertently omitted the labeling of "H" in FIGURES 3-2 to 3-3 as filed, and are submitting herewith replacement FIGURES 3-1 to 3-8 to insert the bold "**H**". Support for the amendment can be found in Figure 2-2 of the Provisional Application No. 60/429,518, which is incorporated by reference under § 1.57(a). Support for the amendment can also be found in Paragraph [0098] of the instant application, where it describes that the bold "**H**" corresponds to H75 of Bac31A8. In Figures 3-1, because of the 6-amino acid gap in the alignment of BAC31A8, the conserved H is shown at position 81, which is the 75$^{th}$ amino acid.

No new matter is added in the amendments. The Examiner is requested to enter the amendments.

### Restriction Requirement

The key of this invention is that Applicant has discovered that a mutation in the conserved histidine of a naturally occurring proteorhodopsin provides a proteorhodopsin mutant with improved optical characteristics, i.e. has lower p$K_{rh}$ in comparision with the naturally occurring proteorhodopsin. Such technical feature was not taught or disclosed in any prior art.

The Examiner found Applicants' arguments unpersuasive because the phrase "conserved histidine" was unclear. Applicants have amended Claim 1 to clarify the meaning of the claim. In

view of the claim amendment and the filing of RCE, Applicants respectfully request that the Examiner reconsiders the restriction requirement.

**35 U.S.C. 112, Second Paragraph Rejection**

Claims 1, 4-5, 7-9, 14, 23-27 remain rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The rejection is overcome in view of the claim amendment.

**Conserved histidine residue**

The Examiner states that the phrase "conserved histidine residue" in claim 1 (and its dependent claims 4-5, 7-8, 14) is unclear. Applicants have amended the claim to recite that the conserved histidine is present at the position equivalent to position 75 of SEQ ID NO: 3 when the proteorhodopsin variant is aligned with SEQ ID NO: 3 for a maximum identity.

At Paragraph [0014], the application describes that a conserved histidine residue is at, for example, amino acid position 75 of Bac31A8, or position 77 of Hot75m1, or its equivalent position of a proteorhodopsin variant.

At Paragraph [0154], the application describes methods known in the art for aligning protein sequences. There can be deletions or insertions when two structures are aligned for maximum identity. For example, proteorhodopsin Bac31A8 has only 249 amino acids while proteorhodopsin Hot75m1 has 252 amino acids. Aligning the two sequences shows that Bac31A8 has no residue corresponding to 214 of Hot75M1. Thus, the amino acid sequence of Bac31A8 would appear very different from Hot75m1 unless a gap is recorded between locations 211 and 212 of Bac31A8 (see FIG. 2 for alignment).

As shown in Figure 3, naturally occurring proteorhodopsins have high degree of homology. Applicants have shown "the conserved histidine" in 81 naturally occurring proteorhodopsins. **In the 81 proteorhodopsin sequences shown in Figures 3-1 to 3-8, 57 of them contain only one histidine residue, which is the conserved histidine residue.** The remaining 24 proteorhodopsins (HOT2C02, HOT75m3, medA15_R8_3, medA15_r8ex7, medA15_R8ex9, medA15_r9_3, medA15r10b5, medA15r11b3, medA15r8b3, medA15r8b8,

medA15r8b9, medA15r8ex4, medA15r9b5, medA15r9b7, medA17_r8_11, medA17_r8_15, medA17_R8_6, medA17R9_1, medA19_R8_16, medA19_R8_19, medA19_R8_20, medA19_r9_9, PalB1, and PalE6) contain two histidine residues.

There are only very few histidine residues in the naturally occurring proteorhodopsins, as illustrated in Figures 3-1 to 3-8. **A skilled person in the art can easily compare and align the amino acid sequences of Bac31A8 and any naturally occurring proteorhodopsin or its proteorhodopsin homolog having 90% identity and determine where the conserved histidine is.**

Therefore, the phrase "the conserved histidine" in a naturally occurring proteorhodopsin is not indefinite.

**Having at least 90% identity**

The Examiner states that the phrase "having at least 90% identity" in claim is unclear. Applicants have amended Claim 1 to recite the proteorhodopsin mutant is a proteorhodopsin variant comprising a mutation in a conserved histidine residue, said proteorhodopsin variant is a naturally occurring proteorhodopsin or a proteorhodopsin homolog having at least 90% identity with the naturally occurring proteorhodopsin.

Paragraph [0003] describes that proteorhodopsins are integral membrane proteins isolated from uncultivated marine eubacteria and function as light-driven proton pumps. The naturally occurring proteorhodopsin in Claim 1 is meant to include naturally occurring proteorhodopsins isolated from marine eubacteria.

Paragraph [0154] describes that closeness of relation can be measured by comparing amino-acid sequences. Methods of aligning protein sequences and methods of defining relatedness are described in the application and well known to a person skilled in the art. A skilled person can easily align a naturally occurring proteorhodopsin with its homolog and compare them for percent of identity.

Therefore, the phrase "having at least 90% identity" is not indefinite.

**35 U.S.C. 112, First Paragraph Rejection**

Claims 1, 4-5, 7-9, 14, 23-27 are rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement.

Contrary to what the Examiner has asserted, Applicants did not claim 90% homologs of a homolog. However, to further the prosecution, Applicants have amended the claims to clarify the meaning of the claims.

Naturally occurring proteorhodopsins have known and defined amino acid sequences. Naturally occurring proteorhodopsins have similar amino acid sequences as illustrated by the 81 sequences in Figure 3. Figure 3 also shows the amino acid sequence alignment of 81 naturally occurring proteorhodopsins and the conserved histidine residue. It is not possible for Applicant to list the amino acid sequences of all naturally occurring proteorhodopsins and show the alignments of the sequences to demonstrate the conserved histidine. However, **Applicant has provided a large representative number (81) of species.**

A proteorhodopsin homolog having at least 90% identity with a naturally occurring proteorhodopsin can be made by changing several amino acids of a naturally occurring proteorhodopsin, and testing its ability for undergoing a photocycle containing an "M-state" or "M-like state" (see Paragraph [0108]). This can be easily done by a person skilled in the art.

Therefore, 35 U.S.C. §112, first paragraph of Claims 1, 4-5, 7-9, 14, 23-27 should be withdrawn.

**35 U.S.C. 102(e) Rejection**

Claims 1, 25-27 remain rejected under 35 U.S.C. §102(e) as allegedly being anticipated by La Rosa et al (US2007/0192889).

La Rosa et al. disclose a plant protein that only shows 7.6% sequence identity with the entire sequence of SEQ ID NO: 3, and 22.4% sequence similarity in a short stretch. When the sequence of the La Rosa protein is aligned and compared with the 81 naturally occurring proteorhodopsins in Figure 3, it is clear that the La Rosa protein is **not** a proteorhodopsin variant comprising a mutation in a conserved histidine residue, wherein said proteorhodopsin variant is a naturally occurring proteorhodopsin or a proteorhodopsin homolog having at least 90% identity with the naturally occurring proteorhodopsin.

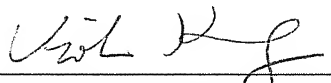
Therefore, the 102(e) rejection of Claims 1, and 25-27 over La Rosa et al should be withdrawn.

## CONCLUSION

Applicants believe that the application is now in good and proper condition for allowance. Early notification of allowance is earnestly solicited.

Date: June 2, 2008

Respectfully submitted,



Viola T. Kung, Ph.D. (Reg. No. 41,131)

HOWREY LLP
2941 Fairview Park Drive, Box 7
Falls Church, VA 22042
Tel: (650) 798-3570
Fax: (650) 798-3600

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          PalE7  TMGKLLLLILG SAIALPSFAA AGGD....LD ISDTVGVSFW LVTAGMLAAT
          RED19  .MGKLLLLILG SVIALPTFAA GGGD....LD ASDYTGVSFW LVTAALLAST
           RED2  .MGKLLLLILG SVIALPTFAA GGGD....LD ASDYTGVSFW LVTAALLAST
          RED23  .MGKLLLLILG SVIALPTFAA GGGD....LD ASDYTGVSFW LVTAALLAST
          RED27  .MGKLLLLILG SVIALPTFAA GGGD....LD ASDYTGVSFW LVTAALLAST
          RED30  .MGKLLLLILG SVIALPTFAA GGGD....LD ASDYTGVSFW LVTAALLAST
           RED4  .MGKLLLRLG SVIALPTFAA GGGD....LD ASDYTGVSFW LVTAALLAST
           RED5  .MGKLLLLILG SVIALPTFAA GGGD....LD ASDYTGVSFW LVTAALLAST
          REDA9  .MGKLLLLILG SVIALPTFAA GGGD....LD ASDYTGVSFW LVTAALLAST
          REDB9  .MGKLLLLILG SVIALPTFAA GGGD....LD ASDYTGVSFW LVTAALLAST
          REDF9  .MGKLLLLILG SVIALPTFAA GGGD....LD ASDYTGVSFW LVTAALLAST
      REDr6a5a14 .MGKLLLLILG SVIALPTFAA GGGD....PD ASDYTGVSFW LVTAALLAST
       REDr6a5a6 .MGKLLLLILG SVIALPTFAA GGGD....LD ASDYTGVSFW LVTAALLAST
      REDr7_1_15 .MGKLLLLILG SVIALPTFAA GGGD....LD ASGYTGVSFW LVTAALLAST
      REDr7_1_16 .MGKRLVILG SVIALPTFAA GGGD....LD ASDYTGVSFW LVTAALLAST
       REDr7_1_4 .MGKLLLLILG SVIALPTFAA GGGD....LD ASDYTGVSFW LVTAALLAST
       REDs3_15  .MGKLLLLILG SVIALPTFAA GGGD....LD ASDYTGVSFW LVTAALLAST
        REDs3_7  .MGKLLLLILG SVIALPTFAA GGGD....LD ASDYTGVSFW LVTAALLAST
       ANT32C12  ..MKLLLILG SAIALPSFAA AGGD....LD ISDTVGVSFW LVTAGMLAAT
        HOT2C02  .......... ....MKVLML NPGD...... ...HVAISFW LISMAMVAAT

                 51                                        H          100
        BAC31A8  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
        BAC40E8  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
        BAC64A5  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
         HOT0m1  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
        HOT75m1  VFFFVERDQV SAKWKTSLAV SGLITGIAFW HYLYMRGVWI DTGDTP....
        HOT75m3  VFFFVERDQV SAKWKTSLTV SGLITGIAFW HYLYMRGVWI DTGDTP....
        HOT75m4  VFFFVERDQV SAKWKTSLTV SGLITGIAFW HYLYMRGVWI DTGDTP....
        HOT75m8  VFFFVERDQV SAKWKTSLTV SGLITGIAFW HYLYMRGVWI DTGDTP....
          MB0m1  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
          MB0m2  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
       MB100m10  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
        MB100m5  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
        MB100m7  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
        MB100m9  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
        MB20m12  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
         MB20m2  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
         MB20m5  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
         MB40m1  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
        MB40m12  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
         MB40m5  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
         MED101  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
         MED102  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGSSP....
         MED106  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
         MED202  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
         MED204  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGSSP....
         MED208  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
          MED25  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGSSP....
          MED26  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
          MED27  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
          MED36  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGSSP....
     medA15_r8_1 VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYLYMRGVWV ETGETP....
     medA15_R8_3 VFFFVERDQV SAKWKTSLTV SGLVTGIAFW HYLYMRGVWI ETGETP....
    medA15_r8ex7 VFFFVERDQV SAKWKTSLTV SGLVTGIAFW HYLYMRGVWI ETGETP....
    medA15_R8ex9 VFFFVERDQV SAKWKTSLTV SGLVTGIAFW HYLYMRGVWI ETGETP....
     medA15_r9_3 VFFFVERDQV SAKWKTSLTV SGLVTGIAFW HYLYMRGVWI ETGETP....
    medA15r10b5  VFFFVERDQV SAKWKTSLTV SGLVTGIAFW HYLYMRGVWI ETGETP....
    medA15r11b3  VFFFIERDRV AAKWKTSLTV SGLVTGIAFW HYLYMRGVWV ETGESP....
    medA15r11b9  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
     medA15r8b3  VFFFIERDRV AAKWKTSLTV SGLVTGIAFW HYMYMRGVWV ETGESP....
     medA15r8b8  VFFIVERDRV SSKWKTSLTV SALVTLIAAV HYFYMRDVWV ATGESP....
     medA15r8b9  VFFFIERDRV SAKWKTSLTV SGLVTGIAFW HYLYMRGVWV DSWTGP.GTG
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insert

**Figure 3-2**

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insert

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                                                    H
medA15r8ex4  VFFIVERDRV SSKWKTSLTV SALVTLIAAV HYFYMRDVWV ATGESP....
medA15r8ex6  VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
 medA15r9b5  VFFFVERDRV AAKWKTSLTV SGLVTGIAFW HYMYMRGVWV ETGESP....
 medA15r9b7  VFFFVERDQV SAKWKTSLTV SGLVTGIAFW HYLYMRGVWI ETGETP....
medA17_r8_11 VFFIVERDRV SAKWKTSLTV SALVTLIAAV HYFYMRDVWV ATGESP....
medA17_r8_15 VFFIVERDRV SAKWKTSLTV SALMTLIAAV HYFYMRDVWV ATGESP....
 medA17_R8_6 VFFFVERDQV SAKWKTSLTV SGLITGIAFW HYLYMRGVWV ETGDSP....
  medA17R9_1 VFFFIERDRV SAKWKTSLTV SGLVTGIAFW HYLYMRGVWV DSWNPETGMG
medA19_R8_16 VFFFVERDQV SAKWKTSLTV SGLITGIAFW HYLYMRGVWI DTGGSP....
medA19_R8_19 VFFFVERDQV SAKWKTSLTV SGLITGIAFW HYLYMRGVWI DTGGSP....
medA19_R8_20 VFFFVERDQV SAKWKTSLTV SGLVTGIAFW HYLYMRGVWI ETGETP....
 medA19_r9_9 VFFFVERDQV SAKWKTSLTV SGLVTGIAFW HYLYMRGVWI ETGETP....
       PalB1 VFFFVERDQV SAKWKTSLTV SGLITGIAFW HYLYMRGVWI DTGDTP....
       PalB2 VFFFVERDQV SAEWKTSLTV SGLITGIAFW HYLYMRGVWI DTGDTP....
       PalB5 VFFFVERDQV SAKWKTSLTV SGLITGIAFW HYLYMRGVWI DTGDTP....
       PalB6 VFFFVERDQV SAKWKTSLTV SGLITGIAFW HYLYMRGVWI DTGDTP....
       PalB7 VFFFVERDQV SAKWKTSLTV SGLITGIAFW HYLYMRGVWI DTGDTP....
       PalB8 VFFFVERDQV SAKWKTSLTV SGLITGIAFW HYLYMRGVWI DTGDTP....
       PalE1 VFFFVERDQV SAKWKTSLTV SGLITGIAFW HYLYMRGVWI DTGDTP....
       PalE6 VFFFVERDQV SAKWKTSLTV SGLITGIAFW HYLYMRGVWI DTGDTP....
       PalE7 VFFFVERDQV SAKWKTSLTV SGLITGIAFW HYLYMRGVWI DTGDTP....
       RED19 VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
        RED2 VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
       RED23 VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
       RED27 VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
       RED30 VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGSSP....
        RED4 VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
        RED5 VFFFVERDRV SAKWKTSLAV SGLITGIAFW HYMYMRGVWI ETGDSP....
        REDA9 VFFFVERDRV SAKWKTSLAV SGLITGIAFW HCMYMRGVWI ETGDSP....
        REDB9 VFSFVERDRV SAKWKTSLTV SGLITGIAFW HYMYMRGVWI ETGDSP....
        REDF9 VFFFVERDRV SAKWKTSLTV SGLITGIAFW HYMYMRGVWI ETGDSP....
  REDr6a5a14 VFFFVERDRV SAEWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
   REDr6a5a6 VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
  REDr7_1_15 VFFFVERDRV SAKWKTSLTV PGLITDIAFW HYMYMRGVWI ETGDSP....
  REDr7_1_16 VFFFVERDRV SAKWKTSLTV SGLVTGIAFW HYMYMRGVWI ETGDSP....
   REDr7_1_4 VFFFVERDRV SAKWKTSLTV PGLITDIAFW HYMYMRGVWI ETGDSP....
    REDs3_15 VFFFVERDRV SAKWKTSLTV PGLVTGIAFW HYMYMRGVWI ETGDSP....
     REDs3_7 VFFFVERDRV SAKWKTSLTV PGLITDIAFW HYMYMRGVWI ETGDSP....
    ANT32C12 VFFFVERDQV SAKWKTSLTV SGLITGIAFW HYLYMRGVWI DTGDTP....
     HOT2C02 AFFFLERDRV AAKWKTSLTV AGLVTGIAAW HYFYMRGVWV ATGDSP....

             101                                                 150
      BAC31A8 ...TVFRYID WLLTVPLLIC EFYLILAAAT NVAGSLFKKL LVGSLVMLVF
      BAC40E8 ...TVFRYID WLLTVPLLIC EFYLILAAAT NVAAGLFKKL LVGSLVMLVF
      BAC64A5 ...TVFRYID WLLTVPLLIC EFYLILAAAT NVAGSLFKKL LVGSLVMLVF
       HOT0m1 ...TVFRYID WLLTVPLLIC EFYLILAAAT NVAAGLFKKL LVGSLVMLVF
      HOT75m1 ...TVFRYID WLLTVPLQMV EFYLILAACT SVAASLFKKL LAGSLVMLGA
      HOT75m3 ...TVFRYID WLLTVPLQMV EFYLILAACT SVAASLFKKL LAGSLVMLGA
      HOT75m4 ...TVFRYID WLLTVPLQVV EFYLILAACT SVAASLFKKL LAGSLVMLGA
      HOT75m8 ...TVFRYID WLLTVPLQMV EFYLILAACT NVAASLFKKL LAGSLVMLGA
        MB0m1 ...TVFRYID WLLTVPLLIC EFYLILAAAT NVAAGLFKKL LVGSLVMLVF
        MB0m2 ...TVFRYID WLLTVPLLIC EFYLILAAAT NVAAGLFKKL LVGSLVMLVF
      MB100m10 ...TVFRYID WLLTVPLLIC EFYLILAAAT NVAGSLFKKL LVGSLVMLVF
       MB100m5 ...TVFRYID WLLTVPLLIC EFYLILAAAT NVAGSLFKKL LVGSLVMLVF
       MB100m7 ...TVFRYID WLLTVPLLIC EFYLILAAAT NVAGSLFKKL LVGSLVMLVF
       MB100m9 ...TVFRYID WLLTVPLLIC EFYLILAAAT NVAGSLFKKL LVGSLVMLVF
       MB20m12 ...TVFRYID WLLTVPLLIC EFYLILAAAA NVAGSLFKKL LVGSLVMLVF
        MB20m2 ...TVFRYID WLLTVPLLIC EFYLILAAAT NVAAGLFKKL LVGSLVMLVF
        MB20m5 ...TVFRYID WLLTVPLLIC EFYLILAAAT NVAGSLFKKL LVGSLVMLVF
        MB40m1 ...TVFRYID WLLTVPLLIC EFYLILAAAT NVAGSLFKKL LVGSLVMLVF
       MB40m12 ...TVFRYID WLLTVPLLIC EFYLILAAAT NVAGSLFKKL LVGSLVMLVF
        MB40m5 ...TVFRYID WLLTVPLLIC EFYLILAAAT NVAAGLFKKL LVGSLVMLVF
       MED101 ...TVFRYID WLLTVPLLIC EFYLILAAAT NVAGSLFKKL LVGSLVMLVF
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**Figure 3-3**